

# Claims

What is claimed is:

1. An assay for detecting, measuring or monitoring the activity or concentration of a protein in a test sample, wherein the protein belongs to a plurality of proteins and the plurality of proteins have similar or overlapping properties towards a plurality of substrates, comprising  
determining the activity or the concentration of the protein in the test sample with a sensitivity coefficient of each substrate for the protein.
2. The assay of claim 1, further comprising  
adding each substrate to test sample aliquots; and  
measuring reaction rates between the protein and each substrate.
3. The assay of claim 1, wherein each sensitivity coefficient is determined from a sensitivity coefficient sample by  
obtaining a plurality of inhibited dilutions of the sensitivity coefficient sample, wherein the plurality of inhibited dilutions comprise a plurality of concentrations of the protein which are partially to completely inhibited;  
exposing each inhibited dilution of the plurality of inhibited dilutions to each substrate;  
measuring the reaction rates between each uninhibited protein in each inhibited dilution and each substrate;  
calculating the relationships between the reaction rates of each uninhibited protein and each concentration of the sensitivity coefficient sample at infinite inhibitor concentration; and  
extracting each sensitivity coefficient of each substrate for each protein from the calculated relationships.
4. The assay of claim 3, wherein the plurality of inhibited dilutions is obtained by obtaining a plurality of dilutions of at least one inhibitor which selectively inhibits a protein belonging to the plurality of proteins;  
obtaining a plurality of dilutions of the sensitivity coefficient sample; and

adding each dilution of the inhibitor to each dilution of the sensitivity coefficient sample.

5. The assay of claim 1, wherein the concentration or activity of more than one protein in a test sample is detected, measured or monitored.

5 6. The assay of claim 1, wherein the plurality of proteins comprise acetylcholinesterase and butyrylcholinesterase.

7. The assay of claim 1, wherein the plurality of substrates is selected from the group consisting of acetylcholine, acetylthiocholine, butyrylcholine, butyrylthiocholine, propionylcholine, and propionylthiocholine.

10 8. The assay of claim 1, wherein the plurality of substrates comprise acetylthiocholine, butyrylthiocholine, and propionylthiocholine.

9. The assay of claim 4, wherein the inhibitor is huperzine-A, tetraisopropyl pyrophosphoramidate, or a combination thereof.

10. An assay for detecting, measuring or monitoring the activity or concentration of acetylcholinesterase, butyrylcholinesterase, or both in a test sample comprising

determining the activity or the concentration of acetylcholinesterase, butyrylcholinesterase, or both in the test sample with sensitivity coefficients of each substrate for acetylcholinesterase and butyrylcholinesterase.

20 11. The assay of claim 10, wherein the plurality of substrates is selected from the group consisting of acetylcholine, acetylthiocholine, butyrylcholine, butyrylthiocholine, propionylcholine and propionylthiocholine.

12. The assay of claim 10, wherein the plurality of substrates comprise acetylthiocholine, butyrylthiocholine, and propionylthiocholine.

25 13. The assay of claim 10, wherein the test sample is a synthetic sample or a natural sample.

14. The assay of claim 10, wherein the natural sample is a tissue, fluid, or a membrane.

15. The assay of claim 10, wherein the sample is blood, serum, lymph, cerebrospinal fluid, breast milk, interstitial or urine.

16. The assay of claim 10, wherein the sample is diaphragm, bone marrow, brain, liver, muscle, adrenal and kidney.

5 17. The assay of claim 10, further comprising  
adding each substrate to test sample aliquots;  
measuring the reaction rates between acetylcholinesterase and each substrate;  
and measuring the reaction rates between butyrylcholinesterase and each  
substrate.

10 18. The assay of claim 10, wherein the sensitivity coefficients are  
determined from a sensitivity coefficient sample by  
obtaining a plurality of dilutions of at least one inhibitor which selectively  
inhibits either acetylcholinesterase or butyrylcholinesterase;  
obtaining a plurality of dilutions of the sensitivity coefficient sample;  
15 adding each dilution of the inhibitor to each dilution of the sensitivity  
coefficient sample to obtain a plurality of inhibited sensitivity coefficient samples;  
exposing each inhibited sensitivity coefficient sample to each substrate;  
measuring the reaction rates between acetylcholinesterase and each substrate;  
measuring the reaction rates between butyrylcholinesterase and each substrate;  
20 calculating the relationship between the reaction rates of acetylcholinesterase  
and each concentration of the sensitivity coefficient sample at infinite inhibitor  
concentration;

calculating the relationships between the reaction rates of  
butyrylcholinesterase and each concentration of the sensitivity coefficient sample at  
25 infinite inhibitor concentration; and

extracting each sensitivity coefficient of each substrate for  
acetylcholinesterase and butyrylcholinesterase from the calculated relationships.

19. The assay of claim 18, wherein the inhibitor is huperzine-A,  
tetraisopropyl pyrophosphoramidate, or a combination thereof.

20. The assay of claim 17, wherein measuring the reaction rates comprises utilizing a chromogenic substrate and measuring the absorbance of the reactions.

21. The assay of claim 10, wherein the test sample further comprises an agent which affects the concentration or activity of acetylcholinesterase, butyrylcholinesterase, or both.

22. The assay of claim 21, wherein the agent is removed from the test sample prior to measuring the reaction rates.

23. A method of detecting or confirming whether a subject was exposed to an agent which affects the concentration or activity of acetylcholinesterase, butyrylcholinesterase, or both comprising

obtaining a test sample from the subject;  
measuring the reaction rates between acetylcholinesterase and a plurality of substrates;  
measuring the reaction rates between butyrylcholinesterase and the plurality of substrates; and  
calculating the activity or the concentration of acetylcholinesterase, butyrylcholinesterase, or both with sensitivity coefficients of each substrate for acetylcholinesterase and butyrylcholinesterase.

24. A method of determining the identity of an agent which affects the concentration or activity of acetylcholinesterase, butyrylcholinesterase, or both to which a subject was exposed comprising

obtaining a test sample from the subject;  
measuring the reaction rates between acetylcholinesterase and a plurality of substrates;  
measuring the reaction rates between butyrylcholinesterase and the plurality of substrates; and  
calculating the activity or the concentration of acetylcholinesterase, butyrylcholinesterase, or both with sensitivity coefficients of each substrate for acetylcholinesterase and butyrylcholinesterase; and

comparing the activities or the concentrations with a database of activity and concentration acetylcholinesterase and butyrylcholinesterase profiles for agents which affect the concentration or activity of acetylcholinesterase, butyrylcholinesterase, or both.

- 5           25.     A method of determining the efficacy or monitoring the progress of a treatment regime, wherein a subject is administered a compound which affects the concentration or activity of acetylcholinesterase, butyrylcholinesterase, or both comprising
  - obtaining a test sample from the subject;
  - 10           measuring the reaction rates between acetylcholinesterase and a plurality of substrates;
  - measuring the reaction rates between butyrylcholinesterase and the plurality of substrates;
  - calculating the activity or the concentration of acetylcholinesterase, butyrylcholinesterase, or both with sensitivity coefficients of each substrate for  
15           acetylcholinesterase and butyrylcholinesterase; and
  - monitoring the activities or the concentrations of acetylcholinesterase, butyrylcholinesterase, or both as a function of time of the treatment regime.
- 20           26.     A method of determining whether a subject suffers from a drug sensitivity or a disease which affects the activities or the concentrations of acetylcholinesterase, butyrylcholinesterase, or both comprising
  - obtaining a test sample from the subject;
  - measuring the reaction rates between acetylcholinesterase and a plurality of substrates;
  - 25           measuring the reaction rates between butyrylcholinesterase and the plurality of substrates;
  - calculating the activity or the concentration of acetylcholinesterase, butyrylcholinesterase, or both with sensitivity coefficients of each substrate for acetylcholinesterase and butyrylcholinesterase; and

comparing the activities or the concentrations with a database of activity and concentration acetylcholinesterase and butyrylcholinesterase profiles which are typical of individuals suffering from given drug sensitivities and individuals suffering from given diseases which affect the activities or the concentrations of

5 acetylcholinesterase, butyrylcholinesterase, or both.

27. A method of measuring the concentration of red blood cells in a subject comprising

obtaining a test sample from the subject;

measuring the reaction rates between acetylcholinesterase and a plurality of

10 substrates;

measuring the reaction rates between butyrylcholinesterase and the plurality of substrates;

calculating the activity or the concentration of acetylcholinesterase, butyrylcholinesterase, or both with sensitivity coefficients of each substrate for

15 acetylcholinesterase and butyrylcholinesterase;

determining a relationship between standard concentrations of red blood cells and the activities or the concentrations of acetylcholinesterase, butyrylcholinesterase, or both; and

using the relationship to calculate the concentration of red blood cells of the

20 sample.

28. A method of screening for a candidate compound which affects the concentration or activity of acetylcholinesterase, butyrylcholinesterase, or both comprising

obtaining a test sample;

25 measuring the reaction rates between acetylcholinesterase and a plurality of substrates;

measuring the reaction rates between butyrylcholinesterase and the plurality of substrates;

calculating the activity or the concentration of acetylcholinesterase,  
butyrylcholinesterase, or both with sensitivity coefficients of each substrate for  
acetylcholinesterase and butyrylcholinesterase; and

determining whether the concentration or activity of acetylcholinesterase,  
5 butyrylcholinesterase, or both changes.

29. A device for detecting, measuring or monitoring the activities or  
concentrations of acetylcholinesterase, butyrylcholinesterase, or both in a test sample  
wherein the device measures the reaction rates between acetylcholinesterase and  
butyrylcholinesterase and at least two substrates; and calculates the activities or the  
10 concentrations of acetylcholinesterase, butyrylcholinesterase, or both with sensitivity  
coefficients of each substrate for acetylcholinesterase and butyrylcholinesterase.

30. The device of claim 26, further comprises a cartridge comprising the  
reagents, buffers, substrates and standards for measuring the reaction rates.

31. A kit for detecting, measuring or monitoring the activities or  
15 concentrations of acetylcholinesterase, butyrylcholinesterase, or both in a test sample  
comprising substrates for acetylcholinesterase and butyrylcholinesterase.

32. The kit of claim 31, further comprising a device for measuring the  
reaction rates between acetylcholinesterase and butyrylcholinesterase and the  
substrates, and calculating the activities or concentrations acetylcholinesterase and  
20 butyrylcholinesterase.

33. The kit of claim 31, wherein the substrates for acetylcholinesterase and  
butyrylcholinesterase include acetylthiocholine, butyrylthiocholine, and  
propionylthiocholine.

34. The kit of claim 31, further comprising a chromogenic substrate.

25 35. A biosensor capable of detecting an agent which affects the  
concentration or activity of acetylcholinesterase, butyrylcholinesterase, or both which  
comprises a known mixture of acetylcholinesterase and butyrylcholinesterase  
immobilized on a support and a sealed chamber containing the known mixture of  
acetylcholinesterase and butyrylcholinesterase.

36. A database of sensitivity coefficients for calculating the activities or the concentrations of acetylcholinesterase, butyrylcholinesterase, or both made by a method comprising

obtaining a plurality of inhibited dilutions of a sensitivity coefficient sample,  
5 wherein the plurality of inhibited dilutions comprise a plurality of concentrations of either acetylcholinesterase or butyrylcholinesterase which is partially to completely inhibited;

exposing each inhibited dilution of the plurality of inhibited dilutions to each substrate in a plurality of substrates for acetylcholinesterase and

10 butyrylcholinesterase;

measuring the reaction rates between acetylcholinesterase and each substrate;  
measuring the reaction rates between butyrylcholinesterase and each substrate;  
calculating the relationship between the reaction rates of acetylcholinesterase

and each concentration of the sensitivity coefficient sample at infinite inhibitor  
15 concentration;

calculating the relationships between the reaction rates of butyrylcholinesterase and each concentration of the sensitivity coefficient sample at infinite inhibitor concentration; and

extracting each sensitivity coefficient of each substrate for  
20 acetylcholinesterase and butyrylcholinesterase from the calculated relationships.

37. The database of claim 36, wherein the plurality of inhibited dilutions is obtained by

obtaining a plurality of dilutions of at least one inhibitor which selectively inhibits either acetylcholinesterase or butyrylcholinesterase;

25 obtaining a plurality of dilutions of the sensitivity coefficient sample; and  
adding each dilution of the inhibitor to each dilution of the sensitivity coefficient sample.